

TABLE 2

Morphological differences of WBC				
WBC type	Ratio nucleus/cytoplasm	Nucleus features	Granulae density	Cell size
Lymphocyte	Medium to low	Round to kidney-shaped	None	Small to medium
Monocyte	High	Kidney-shaped, bulging or segmented nucleus	None	Medium to large
Neutrophil	Low	Band-like nucleus or 3-5 segments	Low (very fine)	Medium
Eosinophil	Low	2-3 segments	High (more evenly sized)	Medium
Basophil	Low	3-4 poorly defined segments	High (unevenly sized)	Medium

[0087] Table 3 shows classification rates using different depth of fields (DOF), as above, and thus achieving different resolution. In both cases only a slice of the WBC is in focus.

TABLE 3

Classification rates of WBC with 40x objective 0.55 NA		
	Illumination @ 530 nm	Illumination @ 650 nm
Basophils	94.87%	92%
Eosinophil	91.73%	75%
Monocytes	80.7%	87%
Neutrophils	74.85%	60%
Lymphocytes	96.99%	90%

[0088] The novelty in the invention with respect to the setup using microchannels and DHM, and further algorithm training, is fourfold:

[0089] First, an image-based label free 5-part differential analysis of white blood cells can be performed as opposed to perming Giemsa staining to label the WBC.

[0090] Second, the use of the deep learning technology provides a rich domain of features to be used in classification as opposed to conventional hematology analyzers that depend mainly on the forward and orthogonal light scattering.

[0091] In deep learning systems, the hidden layers can extract features from the input images. Low level and high level features are extracted based on the composition of the hidden layers. Such features may be as simple as the image intensities and their statistics and can be more complex such as the formation of edges, corners etc.

[0092] Further, a large number of features can be extracted. These features do not necessarily correspond to physical characteristics of each cell but they can play an essential role in differentiating the cells.

[0093] Third, a high leukocyte differentiation accuracy (>75%) can be observed with a DOF <5.7 μm and a lateral resolution limit of <0.6 μm , which sets boundary conditions for the objective NA and the used wavelength to image the cells.

[0094] Last and most important, the leukocytes can be in an in-vivo, native condition, e.g., a room temperature stored EDTA-stabilized blood sample not older than one day. No fixation or staining has to be performed, but leukocytes are imaged directly in blood plasma or an isotonic buffer. This

is a setting which is compatible with high throughput workflow (typically 60 s/sample and a statistic on all WBC from $\sim 2 \mu\text{l}$).

[0095] Most important as compared to prior art is the possibility to discriminate granulocytes (neutrophils, eosinophils and basophils) which usually cannot be achieved with any other label-free method (e.g. 2-photon microscopy, conventional phase contrast microscopy, regular microscopy based on intensity values, hematology analysis by Mie scatter or impedance analysis) at present.

1. A method for marker-free detection of a cell type of at least one cell in a medium, comprising:

flowing a medium comprising at least one cell into a microfluidic device,

obtaining an image of the at least one cell in the microfluidic device by a digital holographic microscopic device, wherein the image is obtained with a depth of field of less than 6 μm , and

determining the cell type of the at least one cell.

2. The method of claim 1, wherein the image is obtained with a lateral resolution of less than 0.6 μm .

3. The method of claim 1, wherein the marker-free detection is carried out while flowing the medium comprising the at least one cell through the microfluidic device.

4. The method of claim 3, wherein the flow in the microfluidic device is at least laminar in a region wherein the image of the at least one cell is obtained.

5. The method of claim 4, wherein the microfluidic device comprises a microchannel in the region wherein the image of the at least one cell is obtained in which the laminar flow is produced by at least one sheath flow.

6. The method of claim 5, wherein surfaces of the microchannel perpendicular to a wavefront of a reference or detection beam of the digital holographic microscopic device are essentially planar.

7. The method of claim 1, wherein a field of view of the digital holographic microscopic device is at most 1.0 times a width of the microfluidic device in a region wherein the image of the at least one cell is obtained.

8. The method of claim 1, wherein the at least one cell is a blood cell.

9. The method of claim 8, wherein the blood cell is a white blood cell.

10. The method of claim 1, wherein determining of the cell type of the at least one cell is carried out using a deep learning network.

11. The method of claim 1, wherein a reference beam and a detection beam of the digital holographic microscopic device are off axis.

12. A device for marker-free detection of a cell type of at least one cell in a medium, comprising:

a digital holographic microscopic device with a depth of field of less than 6 μm ;

a microfluidic device; and

a detection system configured to determine the cell type of the at least one cell.

13. The device of claim 12, wherein the digital holographic microscopic device has a lateral resolution of less than 0.6 μm .